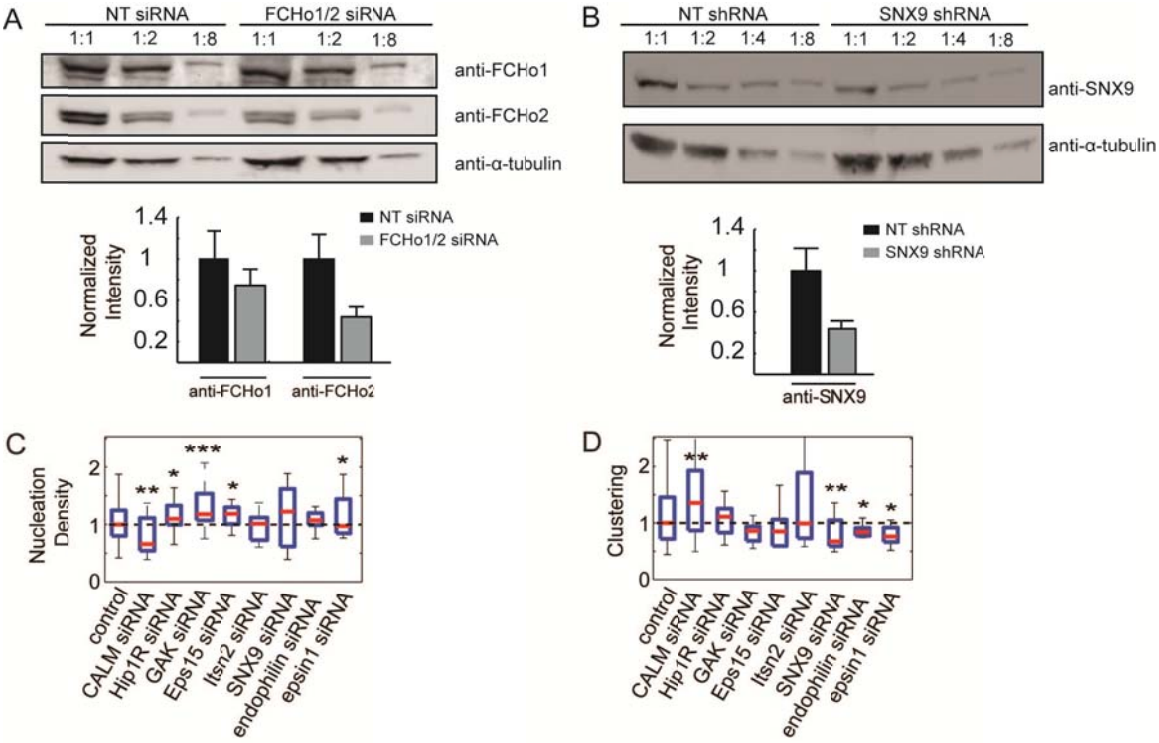
Supplementary Figure for Nunez *et al.*



SUPPLEMENTARY FIGURE 1 Nucleation density and clustering effects of CME accessory proteins

(A) Dilution series of FCHo1, FCHo2, and α-tubulin in cells treated with siRNA against FCHo1 and 2 (FCHo1/2 siRNA) and cells treated with a non–targeting siRNA (NT siRNA). From these dilutions we calculate a 20% and 55% knock-down efficiency (calculated using ImageJ as described in (27)) of FCHo1 and 2, respectively in FCHo1/2 siRNA treated cells relative to the NT siRNA treated cells. The bar graphs show the mean intensities of each dilution series for both FCHo1 and 2 bands. Briefly each band in a dilution series is normalized to the intensity of the corresponding tubulin band. These are then averaged for each dilution series and normalized to the average of the NT siRNA series. (B) Dilution series of SNX9 and actin in cells treated with shRNA against SNX9 (SNX9 shRNA) and cells treated with a non–targeting shRNA (NT shRNA). From these dilutions we calculate a 50% knock-down efficiency in the SNX9 shRNA treated cells relative to the NT shRNA treated cells. The bar graphs show the mean normalized intensities of each dilution series. These are normalized as in (A). (C) Normalized nucleation density and (D) normalized clustering density for cells treated with non-targeting siRNA (n = 30; normalized to group's median), and for cells treated with siRNA against CALM (n = 18), Hip1R (n = 15), GAK (n = 19), Eps15 (n =13), intersectin2 (n = 16; normalized to the median of 48 non-targeting siRNA treated cells), SNX9 (n = 18), endophilin (n = 14), and epsin1 (n = 13) all normalized to non-targeting siRNA treated cells (n = 30), except for the noted exception. * is $P < 0.05$, ** is $P < 0.01$, *** is $P < 0.001$ according to paired t-test (see Methods). All conditions where compared to controls from the same user in order to account for user-to-user variability in acquisition conditions.